REMARKS

The foregoing amendments and these remarks are made in response to the Office Action of October 1, 2003, and the February 19, 2004 telephone interview. Applicants wish to thank Examiners Srivastava and Weber for the courtesy extended to the Applicants at the telephone interview of February 19, 2004.

With respect to the Examiner's objection to the quality of page 2 of the specification, Applicants enclose herewith a replacement page, and certify that the replacement page is identical to page 2 of the application as originally filed. Further, as page 2 relates to background information, no new matter has been introduced.

In the claims, Applicants have cancelled claims 3 and 11, amended claims 1, 2, and 4-10, and added new claims 12 through 16. Accordingly, claims 1, 2, 4-10, and 12-16 are pending herein. The amendments to the claims are discussed below.

With respect to the Examiner's presumption regarding ownership of the invention, Applicants confirm that the subject matter of claims 1 through 16 was commonly owned at the time of invention.

In response to the Examiner's objection to the term "derived" under 35 USC 112 as rendering the claims unclear, Applicants have amended claims 1 and 10 to replace the term "derived" with the term "obtained". Applicants have further specified that the sample is a tissue or cell or body fluid sample for clarity.

For the purpose of clarifying certain details of the subject invention, Applicants provide the following summary of the developmental history of the invention.

Prior to undertaking the research leading to the presently claimed invention, it was not appreciated that any exogenous compound could be acetylated by SSAT, as all acetylated compounds were assumed to be substrates for N-acetyl transferase enzymes NAT1 and NAT2. There was, therefore, no assumption or suggestion in the prior art that SSAT activity could be readily detected or quantified, as the prior art did not teach any specific SSAT substrate or metabolite that could be reliably detected. In the context of this prior knowledge, Applicants have discovered that SSAT has at least one previously unrecognized substrate, namely amantadine. Amantadine is not acetylated by NAT1/NAT2, but is acetylated by SSAT. Therefore, detection of acetylamantadine in a mammalian sample has now been determined to be a reliable marker for SSAT activity.

In the Office Action, the Examiner's has objected under 35 USC 112 to claim 1, alleging that more steps must be recited. The Examiner has stated that "Simply measuring the amount of an acetylated product does not indicate that the product was acetylated by said enzyme". Applicants respectfully disagree. Applicants' invention is based on the finding that SSAT not only acetylates spermine and spermidine (which is the natural substrate

for SSAT as disclosed in the prior art, but which acetylation is difficult to quantify), but SSAT also acetylates non-spermine/spermidine substrates. Amantadine is an example of one such substrate. Applicants have shown that amantadine (a non-spermine/spermidine SSAT substrate) is not acetylated by any enzyme other than SSAT, and therefore acetylated amantadine detected in a biological sample must have been acetylated by the SSAT enzyme. Accordingly, Applicants submit that the only step necessary to detect SSAT activity is the detection of an acetylated non-spermine/spermidine SSAT substrate in a biological sample.

However, for clarity and in view of the Examiner's comments that addition of a step of correlating the detection of acetylated substrate to SSAT would satisfy the requirements of 35 USC 112, Applicants have amended claim 1 to include such a step.

With respect to the Examiner's objections under 35 USC 102/103 with reference to Alvaro [sic, Bras] et al (Can J Physiol Pharmacol, 1998, Vol 76, pp.701-706), it is believed that the Examiner's objections are moot in view of the foregoing amendments. However, for completeness, Applicants offer the following remarks.

This paper was published by the Applicants, and is not suggestive of the presently claimed invention. The Bras publication demonstrated that two other enzymes (NAT1 and NAT2), which were widely known to acetylate drug molecules, were unable to acetylate amantadine. The paper gives no data concerning the SSAT

enzyme. As a result, there is no direction or teaching within this reference that would lead a worker of ordinary skill to recognize amantadine as a substrate to determine the activity of SSAT. In other words, it is difficult to imagine how a publication which does not specifically identify the SSAT enzyme would be suggestive of a methodology to detect that enzyme, or to determine the activity of that enzyme.

Furthermore, during the February 19, 2004 telephone interview with the Examiner, it was indicated that this objection was raised due to the recitation of only one method step in claim 1, namely assaying a sample for the level of a non-spermine/spermidine SSAT substrate. The Examiner alleges that the previously recited single step of claim 1 was inherently disclosed by the Bras publication. Applicants' amended claim 1 now recites steps that are not disclosed by the Bras publication, and therefore submits that the Bras et al reference cannot anticipate or render obvious the presently claimed subject matter.

Furthermore, on the basis of the foregoing, the information in Koppel and Morgan, in combination with the Bras et al teachings, could not lead a worker skilled in the art to the claimed subject matter, particularly as neither Koppel nor Morgan disclose any SSAT substrate that can be reliably used to determine the level of SSAT activity in a mammal.

In view of the above, it is submitted that claim 1 and each of its dependent claims are patentably distinguished over the prior art of record.

Applicants also offer the following remarks in respect of the additional claim amendments.

Claim 10 is directed to a specific embodiment of claim 1, namely that in which the non-spermine/spermidine SSAT substrate is a non-diaminopropane compound.

New claim 12 is directed to methods which employ specific means of measuring acetylated substrate, and is supported in the description at page 7, lines 20 - 22.

New claims 13 - 15 are directed to a method for determining SSAT activity in a sample, and is supported in the description at on pages 7 and 10.

New claim 16 is directed to a preferred embodiment of the method of the invention.

Applicants also file herewith an Information Disclosure Statement identifying the references cited in the International Search Report by the European Examiner in respect of Applicant's corresponding PCT application. Notably, the Bras reference was also identified by the European Examiner, but was indicated as a "document defining the general state of the art which is not considered to be of particular relevance".

In view of the foregoing, favourable reconsideration of the application is requested.

The present application well-describes and claims patentable subject matter. The favorable action of allowance of the pending claims and passage of the application to issue is respectfully requested.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), Applicants respectfully petition for a three (3) month extension of time for filing a response in connection with the present application. The required fee of $475.00 is attached hereto.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell (Reg. No. 36,623) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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0030-0206P

Attachment(s): Substitute page 2 of the Specification

(Rev. 02/12/2004)

acetylator phenotype. This observation suggested that NAT2 was not the acetyltransferase enzyme that catalyzed this conjugation reaction (Sitar *et al.*, 1991). Further still, Bras et al. (1998) reported amantadine acetylation may be effected by acetyltransferases other than N-acetyltransferase 1 (NAT1) or NAT 2.

Spermidine/Spermine Acetyltransferase (SSAT)

Spermidine/spermine N^1-acetyltransferase (SSAT), is ubiquitously distributed in mammalian tissues and plays a role in catabolism and elimination of polyamines from cells (Cohen, 1998; Morgan, 1998). However, in normal or uninduced mammalian tissues SSAT is present at very low levels (Casero & Pegg, 1993; Cohen, 1998). SSAT is an inducible enzyme that catalyzes the transfer of an acetyl group from acetyl-coenzyme A to the aminopropyl moiety of polyamines. This action by SSAT facilitates polyamine degradation, excretion, cycling and/or intracellular cycling (Casero & Pegg, 1993). In this manner SSAT participates in the maintenance of polyamine homeostasis in mammalian cells.

Regulation of SSAT

Induction of SSAT can be caused by different drugs, growth factors, polyamines, polyamine analogues, toxic substances, hormones, and physiological stimuli (Casero & Pegg, 1993). All could cause induction, but the induction occurs at different times for each individual compound. The regulation of SSAT expression occurs at the levels of transcription, mRNA stability, mRNA translation and protein stability (Fogel-Petrovic *et al.*, 1997).

The SSAT gene contains a polyamine responsive element located in a region that occurs at –1522 to –1492 with respect to the SSAT transcriptional start site (Wang *et al.*, 1998). Within this 31 base pair sequence, the polyamine response element was identified as a 9 base pair sequence. The polyamine response element mediates transcriptional induction of SSAT by the polyamine analogue N^1,N^{12}-bis(ethyl)spermine or natural polyamines (Wang *et al.*, 1998).

SSAT, the rate-limiting enzyme in the catabolic pathway plays a regulatory role in maintaining spermidine and spermine homeostasis. It has been estimated that less than 1000 molecules of SSAT are present in a rat hepatocyte, compared to 60,000 molecules in an induced cell (Matsui & Pegg, 1981; Pegg *et al.*, 1982). The induction of mammalian tissues by the various inducers